

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
24 July 2003 (24.07.2003)

PCT

(10) International Publication Number
WO 2003/060152 A3

(51) International Patent Classification⁷: C12N 15/54, 9/22

(21) International Application Number:
PCT/US2003/000542

(22) International Filing Date: 9 January 2003 (09.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/347,403 10 January 2002 (10.01.2002) US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE,
SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for the following desig-
nations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC,
EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL,
PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT,
TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent
(GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG)

Published:

— with international search report

(88) Date of publication of the international search report:
26 February 2004

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: ALTERATION OF RESTRICTION ENDONUCLEASE SPECIFICITY BY GENETIC SELECTION

(57) Abstract: Methods and compositions are provided for altering the DNA recognition and cleavage characteristics of an endonu-
clease without prior knowledge of the endonuclease's three-dimensional structure and/or amino acid residues responsible for activity
and/or specificity. Methods include subjecting a mutagenized endonuclease gene library to a genetic selection in prokaryotic cells
which tolerate the expression of mutated endonuclease and where the endonuclease is active and determining the altered recogni-
tion-site specificity for the endonuclease.

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From the INTERNATIONAL SEARCHING AUTHORITY

To:
HARRIET M. STRIMPEL
PATENT COUNSEL
NEW ENGLAND BIOLABS, INC.
32 TOZER ROAD
BEVERLY, MA 01915

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NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

Date of Mailing (day/month/year) 01 DEC 2003	
Applicant's or agent's file reference NEB-198-PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US03/00542	International filing date (day/month/year) 09 January 2003 (09.01.2003)
Applicant NEW ENGLAND BIOLABS, INC.	

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34, chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.
3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90 bis.1 and 90 bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer <i>Charles L. Patterson, Jr.</i> Charles L. Patterson, Jr. Telephone No. 703-308-0196
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Form PCT/ISA/220 (April 2002)

(See notes on accompanying sheet)

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference NEB-198-PCT	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US03/00542	International filing date (<i>day/month/year</i>) 09 January 2003 (09.01.2003)	(Earliest) Priority Date (<i>day/month/year</i>) 10 January 2002 (10.01.2002)
Applicant NEW ENGLAND BIOLABS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the Report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (See Box II).

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the abstract,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. _____



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/00542

A. CLASSIFICATION OF SUBJECT MATTER												
IPC(7) : C12N 15/54, 9/22												
US CL : 435/471, 199, 193												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/471, 199, 193												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Computer search - CAPLUS, EAST												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
A, E	EP 1 179 596 A1 (FERMENTAS AB) 13 February 2002 (13.02.2002).	1-17										
A	LANIO, T, et al. Towards The Design Of Rare Cutting Restriction Endonucleases: Using Directed Evolution To Generate Variants Of EcoRV Differing In Their Substrate Specificity By Two Orders Of Magnitude. J. Mol. Biol. 1998, Vol. 283, pages 59-69.	1-17										
A	HEITMAN, J, et al. Mutants Of The EcoRI Endonuclease With Promiscuous Substrate Specificity Implicate Residues Involved In Substrate Recognition. EMBO J., 1990, Vol. 9, No. 10, pages 3369-3378.	1-17										
A, E	SAMUELSON, J.C., et al. Directed Evolution Of Restriction Endonuclease BstYI To Achieve Increased Substrate Specificity. J. Mol. Biol. 2002, Vol. 319, pages 673-683.	1-17										
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.												
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Date of the actual completion of the international search 12 November 2003 (12.11.2003)		Date of mailing of the international search report 01 DEC 2003										
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230		Authorized officer <i>Charles L. Patterson, Jr.</i> Charles L. Patterson, Jr. Telephone No. 703-308-0196										

Form PCT/ISA/210 (second sheet) (July 1998)

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